AMENDMENTS TO THE SPECIFICATION

Please the specification as indicated below:

At page 2, lines 24-28:

In one aspect, this invention is directed to a bovine adipocyte polypeptide (i.e. the bovine leptin protein) substantially free of other bovine polypeptides, or a functional derivative thereof. The present invention includes a bovine adipocyte polypeptide consisting essentially of at least about 8 amino acids of the amino acid sequence depicted in FIGS. 1 and 3-5 (SEQ ID NO: 4) ~~(SEQ ID NOS: 4, 7 and 8)~~, or a functional derivative thereof.

At page 2, line 29, to page 3, line7:

The present invention is also directed to a single or double stranded DNA or an RNA molecule (and their respective allelic variants) consisting essentially of a nucleotide sequence that encodes the above polypeptide, the DNA or RNA molecule being substantially free of other bovine DNA or RNA sequences or in other words, isolated or an isolate. The DNA molecule is preferably a single or double stranded DNA molecule having a nucleotide sequence consisting essentially of at least about 20 nucleotides of the nucleotide sequence depicted in FIGS. 1 and 2 (~~SEQ ID NO: 1~~ SEQ ID NO:3) or a sequence complementary to at least part of the nucleotide sequence depicted in FIGS. 1 and 2 (~~SEQ ID NO: 1~~ SEQ ID NO:3), or an allelic variant thereof, substantially free of other bovine DNA sequences. The RNA molecule is preferably an mRNA sequence encoding the above bovine adipocyte polypeptide, or a functional derivative thereof.

At page 4, lines 15-16:

FIG. 1 depicts the bovine leptin cDNA nucleotide sequence (top) (SEQ ID NO:3) and predicted amino acid sequence (bottom) (SEQ ID NO:4) for the coding region minus the secretory signal.

At page 4, lines 17-18:

FIG. 2A shows a comparison of the bovine leptin cDNA nucleotide sequence (SEQ ID NO:3) with the human nucleotide sequence (SEQ ID NO:5).

At page 4, lines 19-20:

FIG. 2B shows a comparison of the bovine leptin cDNA nucleotide sequence (SEQ ID NO:3) with the murine nucleotide sequence (SEQ ID NO:6).

At page 4, lines 21-22:

FIG. 3A shows a comparison of the predicted bovine leptin amino acid sequence (SEQ ID NO: 4) with the human leptin amino acid sequence (SEQ ID NO: 7).

At page 4, lines 23-24:

FIG. 3B shows a comparison of the predicted bovine leptin amino acid sequence (SEQ ID NO: 4) with the murine leptin amino acid sequence (SEQ ID NO: 8).

At page 4, lines 27-28:

FIG. 5A shows a comparison of the actual N-terminal bovine leptin amino acid sequence (SEQ ID NO:4) ~~(SEQ ID NO:7)~~ with the human leptin amino acid sequence (SEQ ID NO:7).

At page 4, lines 29-30:

FIG. 5B shows a comparison of the actual bovine leptin amino acid sequence (SEQ ID NO:4) ~~(SEQ ID NO:8)~~ with the murine leptin amino acid sequence (SEQ ID NO:8).

At page 6, lines 4-11:

The polypeptide of this invention has an amino acid sequence as depicted in FIGS. 1 and 3-5 (SEQ ID NO:4) ~~(SEQ ID NOS:4, 7 and 8)~~. Also intended within the scope of the present invention is any polypeptide having at least about 8 amino acids present in the above-mentioned sequence. Sequences of this length are useful as antigens and for making immunogenic conjugates with carriers for the production of antibodies specific for various epitopes of the entire protein. Such polypeptides are also useful in screening such antibodies and in the methods of the present invention directed to detection of the leptin protein in biological samples. It is well-known in the art that polypeptides of about 8 amino acids are useful in generation of antibodies to larger proteins of biological interest.

At page 16, line 19, to page 17, line 4:

The single-stranded bovine cDNA pool was used as a template to amplify bovine leptin cDNA in a PCR reaction with synthetic DNA primers based on the published

mouse leptin cDNA sequence. Two pairs of oligonucleotide degenerate primers specific for the human and murine leptin gene were designed and synthesized (DNA International, Lake Oswego, OR). The primers were designed to amplify the coding region of the bovine leptin gene, excluding the secretory signal at the 5'-terminal of the coding region). The forward primer has a sequence of 5'-GGA TCC GGT CTC AGG CCG TGC CYA TCC ARA AAG TCC-3' (contains a BsaI site) (SEQ ID NO:1), and the reverse primer has a sequence of 5'-GAA TTC AGC GCT GCA YYC AGG GCT RAS RTC-3'(contains a Eco47III site) (SEQ ID NO:2), where R=(A,G), S=(C,G), Y=(C,T). PCR was performed using the following conditions: 1x PCR buffer, 1.5 mM $MgCl_2$, 1 mM primers, 0.2 mM dNTPs and 5 units of Taq polymerase per 100-µl reaction. A total of 32 cycles were run with following cycling conditions: 94C., 1 min; 55C., 1.5 min; and 72C., 1.5 min. After running the PCR product on a 1% agarose gel, a band of 449 base pairs was obtained from the PCR-amplified bovine single-stranded cDNA as depicted in FIG. 6. Specifically, lane 1 of FIG. 6 contains the 449 base pair bovine leptin cDNA, lane 2 contains the pASK75 vector DNA, and lane 3 contains standard 100 base pair ladder. The size of the PCR product was consistent with the predicted size of the coding region of the bovine leptin gene. This PCR product was verified in a secondary PCR procedure.

At page 18, lines 7-22:

Sequencing of the insert DNA (both sense and antisense strands) was performed by a commercial laboratory (National Bioscience, Inc.) using the standard Sanger's dideoxy-nucleotide method. Briefly, the PCR product containing the 449 bp band was separated on a 1% low-melting-agarose gel. The 449 bp band was cut from the gel, further purified using a Genecleaning kit (Bio101, Inc, Vista, Calif.), and submitted for sequencing. The sequences were then compared with the Genebank and other databases using the GCG software. The sequence data confirm that the 449 bp product from two independent clones shares approximately 87.6% homology with the human leptin cDNA (FIG. 2A, ~~SEQ ID NO:3~~ SEQ ID NO:5) and 84.9% with the mouse leptin cDNA (FIG. 2B, SEQ ID NO: 6 ~~SEQ ID NO:3~~). The predicted amino acid sequence also shares approximately 87% homology with the human leptin protein (FIG. 3A, ~~SEQ ID NO:4~~ SEQ ID NO: 7) and approximately 86.3% homology with the murine leptin protein (FIG. 3B, SEQ ID NO:8 ~~SEQ ID NO:4~~). Moreover, a portion of the predicted amino acid sequence was confirmed through amino terminal sequencing. Specifically, 30 amino acids comprising the N-terminal sequence have been obtained (FIG. 4, SEQ ID NO:4). The actual amino acid sequence (i.e., the N-terminal sequence (FIG. 4, SEQ ID NO:4)) shares approximately 100% homology with the human leptin protein (FIG. 5A, SEQ ID NO:7), and approximately 100% homology with the murine leptin protein (FIG. 5B, SEQ ID NO:8).

At page 21, line 27, to page 22, line 10:

Reverse Transcription and Polymerase Chain Reaction Amplification. Subcutaneous adipose tissue was obtained by surgical biopsy from the tail-head depot of a gestating cow using local anesthesia. Total RNA was extracted using a modified method based on Chomczynski and Sacchi's acidic guanidine thiocyanate extraction (Chirgwin, J. J., A. E. Przbyla, R. J. MacDonald, and W. J. Rutter, 1979, Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease, Biochemistry 18:5294-5299). Poly A^+ RNA was purified using oligo (dT) cellulose mini-columns purchased commercially. For the reverse transcription reaction, an oligo d(T).sub.12-18 primer and 2 μg poly A^+ RNA were used. In the subsequent polymerase chain reaction (PCR) amplification, the following primer sequences were used: 5'-GAA TCC GGT CTC AGA CCG TGC CUA TCC ARA AAG TCC-3' (sense) (SEQ ID NO: 1) and 5'-GAA TTC AGC GCT GCA YYC AGG GCT RAS RTC-3' (antisense) (SEQ ID NO: 2), where R=A, G; Y=C, T; S=C, G. These primer sequences contain restriction sites (Bsa 1 and Eco47III, BamH I and EcoR I) for subsequent cloning, expression, and insert removal. The PCR protocol was as follows: first cycle, 95 C., 3 min; 52 C. 1 min; 72 C. 1 min; 4 cycles, 94 C., 45 sec; 52 C., 45 sec; 72 C., 1 min; 30 cycles, 94 C., 45 sec; 55 C., 1 min; 72 C., 1 min.